Rhodora

JOURNAL OF THE
NEW ENGLAND BOTANICAL CLUB

Vol. 79 January, 1977 No. 817

AN ANALYSIS OF POPULATIONS FORMED BY HYBRIDIZATION BETWEEN PHYLLODOCE EMPETRIFORMIS AND P. GLANDULIFLORA (ERICACEAE)

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Phyllodoce is a genus of seven or eight arctic-alpine subshrubs that are distributed primarily in Asia and North America. Two of the species, *P. empetriformis* (J. E. Smith) D. Don and *P. glanduliflora* (W. J. Hooker) Coville, range in the mountains from Alaska to northern California and Wyoming (Figure 1). Over this region *P. glanduliflora* grows in alpine habitats (usually above 1950 m elevation) which are subject to full insolation and seasonally severe fluctuations in temperature and soil moisture. Populations of *P. empetriformis* are more commonly found at lower elevations (ca. 1500–2000 m) in scattered subalpine forests where climatic conditions are milder. At many sites, however, the distribution of the latter extends into the alpine-subalpine ecotone. Here the two species of *Phyllodoce* grow intermixed and hybridization often occurs. From the intermixed populations an F_1 hybrid — *P.* × *intermedia* (Hook.) Rydb. — was described in 1834 by Hooker (as *Menziesia intermedia* Hook.). Later, Rydberg (1900) recognized a backcross to *P. glanduliflora* as *P. hybrida* Rydb. Camp (1939) observed that the hybridizing populations were morphologically complex "hybrid swarms" and stated that "they [the hybrids] are so numerous and variable that I feel it would be an unnecessary burden upon taxonomic literature to attempt definitive descriptions of the many variants of which I have knowledge." Although precise measurements were not attempted, the formal descriptions and Camp's subsequent evaluation imply that the hybrid populations are typical Andersonian "hybrid swarms"

(Anderson, 1949) with relatively large numbers of F_1s, subsequent filial generations, and backcrosses to both parental species. After examination of herbarium material from the northwestern United States and cursory investigation of a single hybridizing stand at Trapper Peak in Montana, my impressions were that the "swarms" were of simple constitution. The preliminary data suggested that the populations consisted largely of F_1s with very rare advanced filial and/or backcross generations. This investigation was initiated to provide a more accurate account of population structure and gene flow in the hybrid stands.

MATERIALS AND METHODS

For purposes of analysis, 14 populations were sampled (Table 1). Ten of these were either populations of single species or intermixed stands of both species in which no hybrids could be found. Four populations contained both hybrids and parents. A total of 288 plants was examined of which 197 were from hybridizing stands. In addition, specimens from several herbaria (see ACKNOWLEDGMENTS) in the United States and Canada were inspected.

Each individual from a sample was measured for five floral characteristics (Table 2). From these data pictorialized scatter diagrams were prepared (Figures 2, 5, & 6). All measurements were made on dried, pressed materials. The voucher specimens are on deposit in MONTU.

Each plant was examined for flavonoid constituents. The chromatographic and analytical techniques were in general those employed by Harborne (1967) and by Mabry, *et al.* (1970). The flowers and leaves of each plant were extracted separately for 24 hours in 85% aqueous methanol. Extracts of each organ were developed two dimensionally on Whatmann 3MM paper using tertiary butyl alcohol: acetic acid: water (3:1:1 v/v/v) and 15% aqueous acetic acid respectively.

The flavonoids were isolated for further analysis by eluting each compound obtained as a spot on paper with spectral methanol. Each compound was partially characterized by ultraviolet spectral analyses in diagnostic reagents and subsequently hydrolyzed for one hour in 2N HCl to remove the sugars. The aglycone was extracted from the hydrolysis mixture with ethyl acetate, subjected to ultraviolet spectral analysis, and co-chromatogrammed

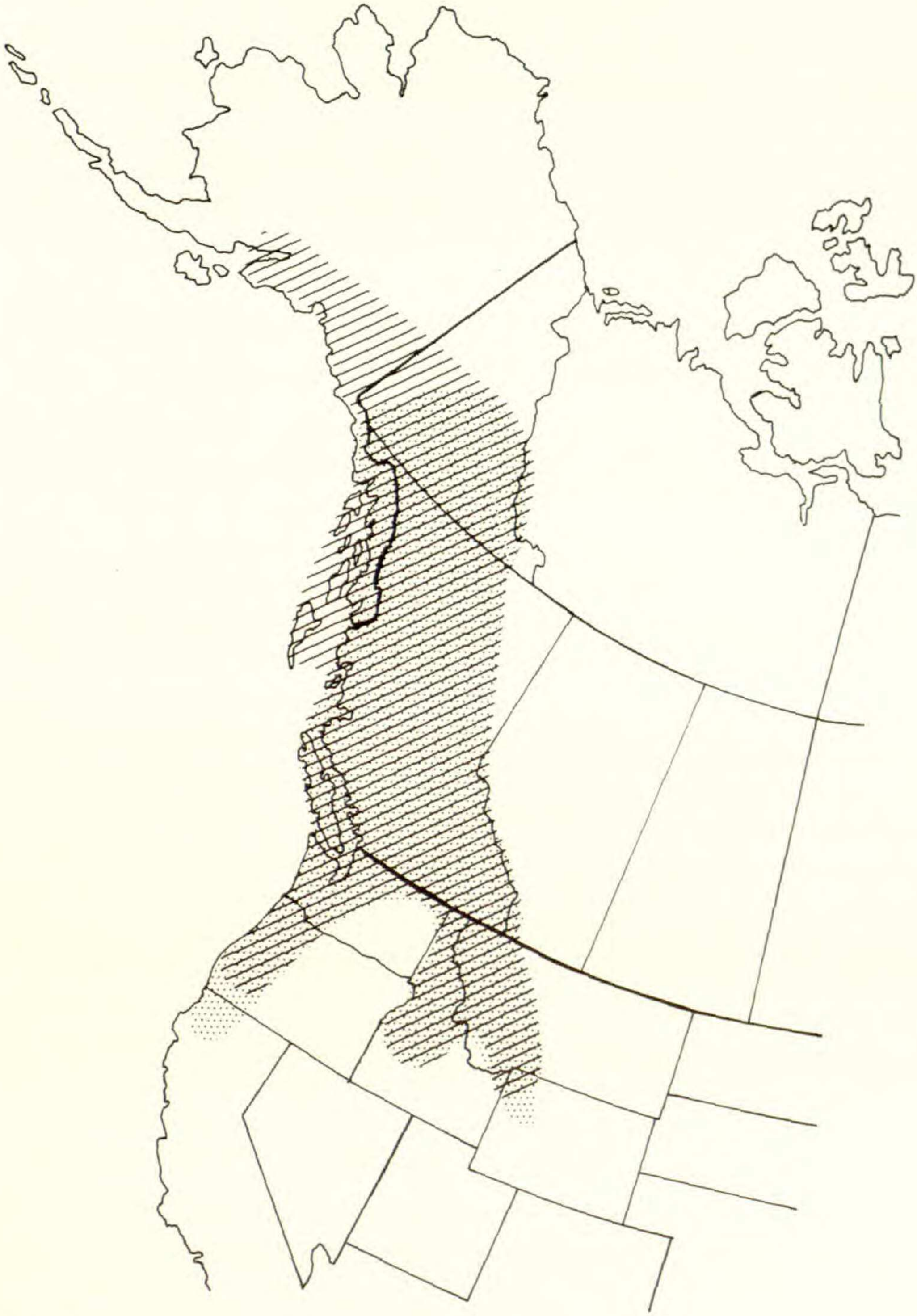


Figure 1. Distribution of two species of *Phyllodoce*. Stippled area, *P. empetriformis;* diagonal lines, *P. glanduliflora.*

Table 1. **Populations Sampled**[1]

Montana

RAVALLI CO.; Bitterroot Mtns., Bitterroot Natl. Forest:
Twin Lakes, ca. 2100 m, 30 June 1973, *Watson 951* (8 plants).
*Trapper Peak, 2820 m, 12 July 1973, *Watson 958* (28 plants).
St. Mary's Peak, 2800 m, 7 July 1973, *Schaack 862* (19 plants).
DEERLODGE CO.; Anaconda-Pintlar Mtns., Deerlodge Natl. Forest:
*Storm Lake, 2490 m, 19 July 1973, *Watson 976* (49 plants).
Goat Flat, 2790 m, 19 July 1973, *Watson 982* (10 plants).
MISSOULA CO.; Lolo Natl. Forest:
Squaw Peak, 2399 m, 26 July 1970, *Watson 990* (14 plants).

Idaho

IDAHO CO.: Papoose Saddle, Bitterroot Mtns., Clearwater Natl. Forest, ca. 1900 m, 28 July 1973, *Watson 994* (5 plants).
Orogrande Summit, Clearwater Mtns., Nezperce Natl. Forest, ca. 1950 m, 28 July 1973, *Watson 996* (6 plants).
SHOSHONE CO.: Freezeout Saddle, Clearwater Mtns., St. Joe Natl. Forest, 1800 m, 2 August 1973, *Watson 997* (8 plants).

Wyoming

PARK CO.: *Beartooth Pass, Beartooth Mtns., Shoshone Natl. Forest, 3280 m, 4 August 1973, *Watson 1000* (66 plants).

Alberta: *Highwood Pass, Rocky Mtns. Forest, 2180 m, 19 August, 1973, *Watson 1009* (54 plants).
Sunwapta Pass, Banff Natl. Park, 2160 m, 21 August 1973, *Watson 1017* (4 plants).
Bow Pass, Crowsnest Forest, 2010 m, 21 August 1973, *Watson 1016* (12 plants).

British Columbia: Mt. Apex, Manning Provincial Park, 2100 m, 2 September 1973, *Watson 1052* (5 plants).

[1]The number of plants collected at each site appears in parentheses.
*Hybridizing populations.

with authentic compounds on paper using the solvent systems described above.

Plants from each site were measured for pollen viability by staining on a microscope slide in aceto-carmine. Grains with normally symmetrical walls, well-developed cytoplasm, and darkly staining nuclei were scored as viable. Percentage of viability was based on random counts of 500 grains from anthers of one to three flowers per plant.

Chromosome studies were attempted but were largely unsuccessful due, perhaps, to the harsh fixative (modified Carnoy's solution) utilized. A count of $2n = 24$ was obtained from a single individual of *Phyllodoce empetriformis*. One count has been previously reported for *P. glanduliflora* as $2n = 24$ (Taylor & Mulligan, 1968).

OBSERVATIONS AND RESULTS

Morphology. *Phyllodoce empetriformis* and *P. glanduliflora* are relatively uniform species in morphological attributes. Vegetatively the two are virtually indistinguishable. Both are woody subshrubs from 10 to 20 cm in height at alpine elevations (*P. empetriformis* is larger at lower altitudes). In alpine regions, the two species are rhizomatous and form clumps 3 to 15 dm in diameter. Both have glabrate, linear leaves with serrulate margins and an abaxial furrow.

The two species differ markedly in flower structure (Figures 2 & 3). The corolla of *Phyllodoce empetriformis* is campanulate and deep pink to violet. The corolla mouth is open and 4.5–8.0 mm in diameter. The style is long and the stigma is greatly exserted. The sepals are short with obtuse apices. The perianth is glabrous.

Phyllodoce glanduliflora has a dirty yellow to yellow-green, urceolate corolla with a narrow mouth. The style is short and the stigma is included. The sepals are long with acute apices. The perianth is moderately to densely pubescent with glandular hairs.

Plants with intermediate morphology or with recombinations of the above floral characters were found only in sites where the two species grow intermixed. The plants recognized as F_1 hybrids in this study (see DISCUSSION) are enclosed by dotted lines on the scatter diagrams (Figures 5 & 6). These progeny are intermediate in every aspect of floral morphology (Figure 3). They have a near campanulate corolla with a slightly constricted mouth; the corolla is cream pink in coloration. The perianth is sparsely glandular. The sepals are of intermediate length and shape. The styles are of intermediate length. The stigma is only slightly exserted.

Filials subsequent to the F_1 exhibited combinations of quantitative and/or qualitative features found in neither the F_1s nor the parental species. These are designated as A1–A8 in Figures 5 and 6. The backcrosses were morphologically intermediate between the F_1 and recurrent species or closely resembled the recurrent

Table 2. **Floral Features and Symbols for Scatter Diagrams**

Character	P. glanduliflora	Intermediate	P. empetriformis
Style length	1.2-3.5 mm	3.6-4.4 mm	4.5 mm or more
Diameter of corolla mouth	1.0-2.5 mm	2.6-4.4 mm	4.5 mm or more
Perianth pubescence	± densely glandular 	sparsely glandular 	glabrous 
Sepal length	3.5 mm or more 	2.9-3.4 mm 	2.8 mm or less 
Corolla color	yellowish 	cream pink 	deep pink-violet 

parent while retaining features found in the noncurrent species. The backcrosses are designated as B1–B16 in Figures 5 and 6.

Flowering Periods. Flowering in *Phyllodoce glanduliflora* is initiated about the third week in June shortly after the snow melts. The populations reach peak anthesis in mid-July and continue flowering until mid-August. In alpine habitats, *P. empetriformis* begins to flower about one week after *P. glanduliflora* and reaches a zenith in middle to late July, ending in late August. The hybrids commence flowering simultaneously with *P. glanduliflora* in June and continue to do so until mid-August. Thus in July and August there is a broad overlap in which there are numerous mature plants of both parents and hybrids in the areas of sympatry available for pollination.

Pollen Viability. Pollen viability measured from individuals of *Phyllodoce empetriformis* was high, ranging largely from 80 to 100% good pollen (mean pollen viability = 91%; standard deviation = 9.9). Individuals of *P. glanduliflora* likewise produced a high percentage of viable grains (range = 75–99%; mean viability = 90%; standard deviation = 9.9).

Plants recognized here as F_1 hybrids exhibited a broad range of pollen viability (0–52%), having a mean of 35% which is considerably lower than the norm of the parental species. Filial generations subsequent to the F_1 ranged from 6 to 48% good grains. Backcrosses produced 46 to 85% viable pollen.

Flavonoid Chemistry. The two species produce a total of ten major flavonoid constituents representing three molecular classes: flavonols, anthocyanins, and dihydroflavonols (Figure 4). The aglycone and position of sugar attachment of some compounds have been determined. Others have not yet been identified or are identified only to molecular class.

Phyllodoce glanduliflora produces flavonoids 6–10 in both flowers and leaves (Figure 4). *Phyllodoce empetriformis* and the F_1 progeny synthesize all ten molecules in the flowers but only compounds 4–10 are found in the leaves. Also, each individual in a pure or nonhybridizing population produces every major compound listed for the profile of that species. Variation was noted only in the quantitative expression of the major flavonoids and in the sporadic occurrence of minor (i.e., weakly visible) constituents. Recombination of major flavonoids was noted only in some advanced filial segregates and in backcrosses to *P. glanduliflora*.

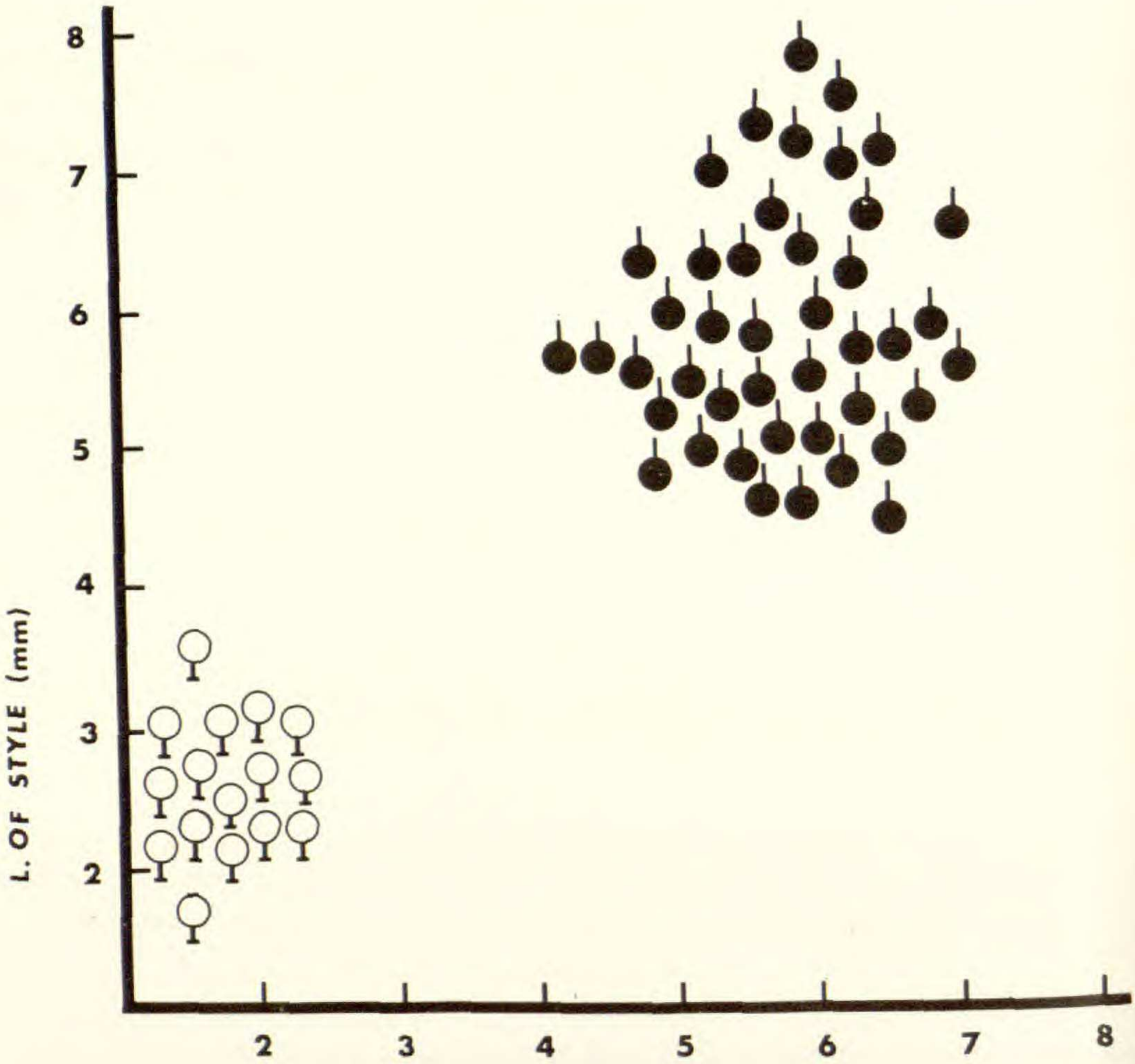


Figure 2. Representative scatter diagram of plants from uncontaminated populations of *Phyllodoce empetriformis* and *P. glanduliflora*. Symbols are listed in Table 2.

In the species of *Phyllodoce* investigated, there appear to be at least two independently segregating linkage groups among the flavonoids: the anthocyanins (spots 1–3) are block-inherited and spots 4 and 5 cohere. Similar linkages are known in flavonoids of *Tragopogon* (Belzer & Ownbey, 1971). While the *Phyllodoce* parents are seemingly homozygous for the loci controlling qualitative expression of these flavonoids, the F_1 is likely heterozygous since neither the anthocyanins nor spots 4 and 5 are produced in *P. glanduliflora*. Recombination of the constituents of each block was not observed in filial generations subsequent to

the F_1 nor in backcrosses. However, four advanced filial segregates and one putative backcross produced the anthocyanins but lacked spots 4 and 5. One backcross synthesized spots 4 and 5 but not the anthocyanins.

DISCUSSION

Flavonoids of *Baptisia* (Turner & Alston, 1959; Alston & Turner, 1962, 1963; McHale & Alston, 1964), *Phlox* (Levin, 1967), *Heterocentron* (Whiffen, 1973), and others have been useful in determining the structure of hybrid populations. In these instances, the parental species had significantly different flavonoid profiles. Each taxon possessed a number of diagnostic compounds that were additive in F_1 progeny and were recombined in some advanced filial segregates and in some backcrosses. In the present study, flavonoid chemistry was of limited value in analyzing the hybrid stands due to the similarity of the parental profiles and to the manner of flavonoid inheritance.

F_1 hybridity in *Phyllodoce* could not be documented on a purely chemical basis because there are no unique molecules contributed to hybrid zygotes by *P. glanduliflora*. The F_1s were identified as having intermediate exomorphic features and low pollen viability. Plants of this generation synthesized all ten flavonoids as expected. Thirty-three individuals were tentatively identified as F_1 progeny (Figures 5 & 6). Probably, a few plants interpreted here as F_1s are actually advanced filial segregates that resemble the F_1 (cf. Anderson, 1949). This probability might account for the broad range of pollen viability observed in the F_1 generation (see RESULTS).

Advanced filial segregates exhibit combinations of morphological characteristics found in neither F_1s nor parents (Figures 5 & 6). Additionally, four plants (A1, A3, A4 and A8 in Figures 5 & 6) have recombinations of flavonoids: the four synthesize the anthocyanins but lack spots 4 and 5. The remaining filial derivatives (A2, A5, A6 and A7 in Figures 5 & 6) elaborate all ten flavonoids. Advanced filials appear to occur infrequently in the hybrid stands. Only eight individuals of this type were found.

Phyllodoce glanduliflora produces no biochemical markers by which to detect backcrossing to *P. empetriformis*. Backcrosses to the latter were recognized by having features of the non-recurrent parent and intermediate pollen viability. All individuals identifield as backcrosses in this direction elaborate the ten flavonoids

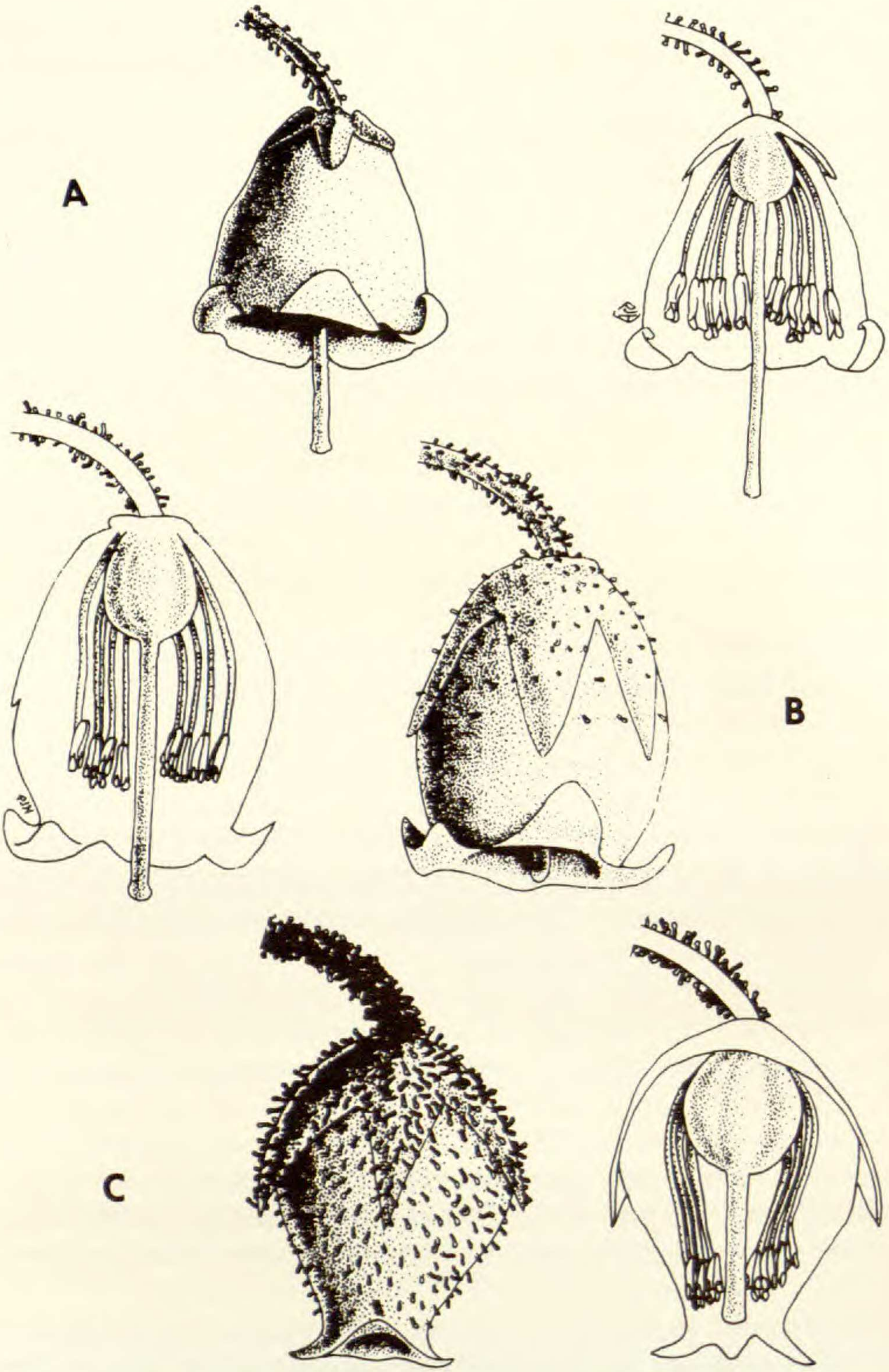


Figure 3. Flowers of *Phyllodoce*. A, *P. empetriformis*; B, F_1 hybrid (*P.* × *intermedia*); C, *P. glanduliflora*.

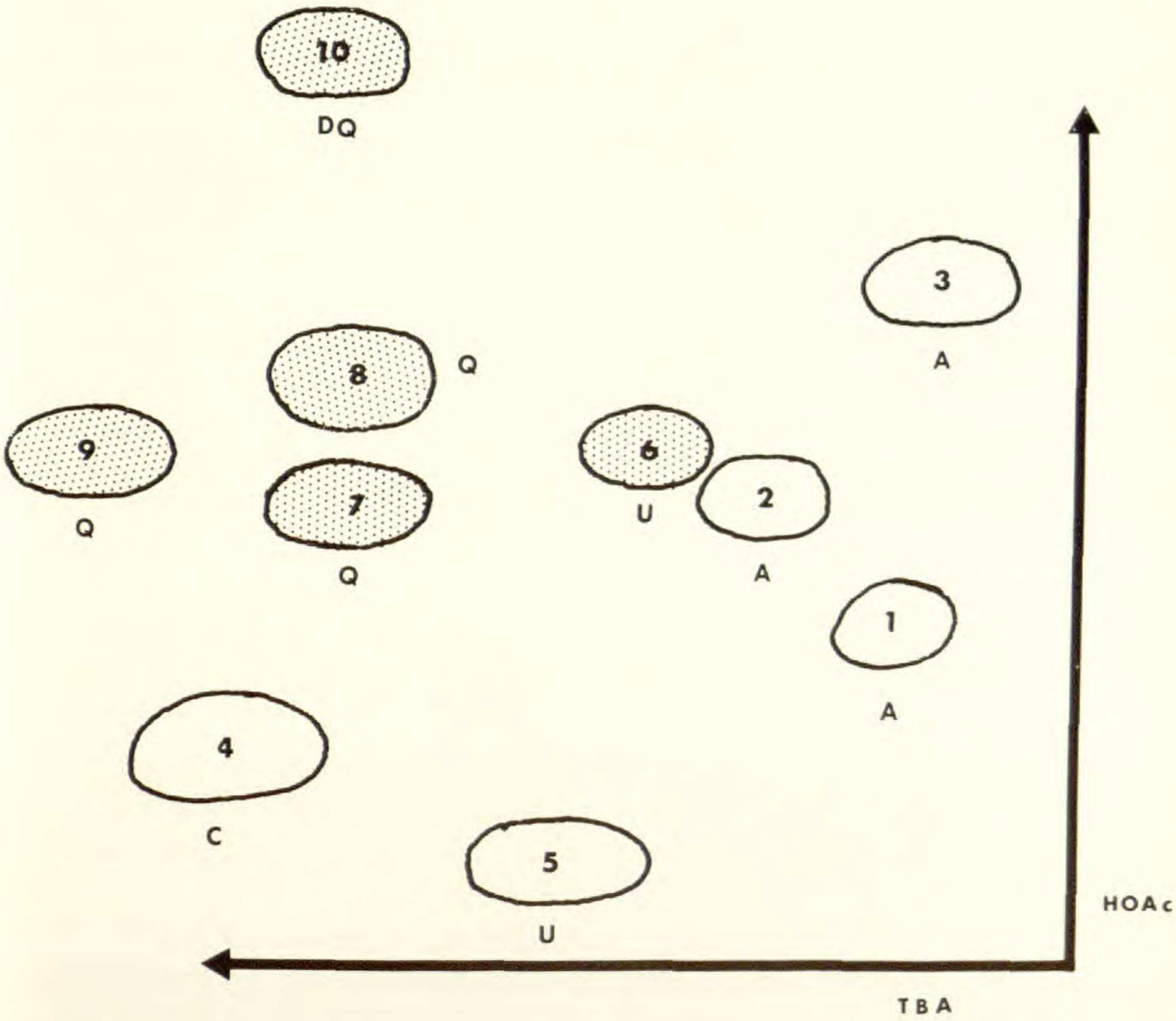


Figure 4. Composite two-dimensional chromatogram of flavonoids in leaves and flowers of *Phyllodoce empetriformis* and *P. glanduliflora*. The stippled spots are synthesized by both species; the remaining compounds are found in *P. empetriformis*. A = anthocyanins; C = caryatin; DQ = dihydroquercetin glycoside; Q = quercetin 3-0-glycosides; U = unknown.

of the recurrent parent. The majority of the backcrosses to *P. empetriformis* fall well within the limits of the taxon in most morphological aspects but have traits attributable to infiltration of genes from *P. glanduliflora* (see B2, B3, B7, B8, B9, B10, B11 and B12 in Figures 5 & 6). These individuals closely resemble the *P. empetriformis* parent but have longer sepals and a few glandular hairs on the perianth. Pollen viability of these plants ranged from 60 to 82%, which is slightly lower than the norm for parental plants. These data are consistent with the interpretation that the

plants are introgressants. Five of the latter (B2, B3, B7, B11 and B12 in Figures 5 & 6) have corollas with hues of pink that are intermediate between the pale pink of the F_1s and the deep pink of the recurrent parent, suggesting segregation of genes which regulate the quantity of anthocyanins produced. Other plants tentatively identified as backcrosses (B4, B5, B6, B13 and B14 in Figure 6) are morphologically intermediate to the F_1 and *P. empetriformis*. Pollen viability for the five plants ranged from 46 to 65% viable pollen. These data suggest that the plants are first generation backcrosses but do not preclude F_1 or subsequent filial hybridity. In this survey, at least eight backcrosses to *P. empetriformis* were recovered.

There were few independently segregating flavonoid markers by which to measure gene flow from *Phyllodoce empetriformis* into *P. glanduliflora*. However, extensive backcrossing to the latter should provide numerous plants resembling *P. glanduliflora* but having flavonoid profiles with one or both *P. empetriformis* linkage groups (i.e., anthocyanins and/or spots 4 and 5) and/or *P. empetriformis* morphological traits. Few plants of this type were recovered. Rather, backcrossing to *P. glanduliflora* seems to occur rarely. Only three plants in the samples may represent backcrosses to the latter taxon (i.e., B1, B15 and B16 in Figures 5 & 6). Two individuals (B1 and B15) are morphologically intermediate between the F_1 and *P. glanduliflora*. B1 produces the anthocyanins but not spots 4 and 5; whereas B15 elaborates all flavonoids. Pollen viability of the plants was 62% and 65% respectively. Although these plants are tentatively identified as backcrosses, they may in fact be filials.

A single individual, B16, was confirmed as a backcross to *Phyllodoce glanduliflora*. This plant resembles *P. glanduliflora* in all morphological aspects but synthesizes the *P. empetriformis* marker compounds 4 and 5. The plant had 85% viable pollen.

The populations created by hybridization between *Phyllodoce empetriformis* and *P. glanduliflora* are apparently simpler than might have been thought previously. Of 197 plants examined from sites of hybridization, 56 appear to be hybrids and their derivatives. The most complex stands are composed largely of parental species and F_1 progeny with relatively small numbers of advanced filial segregates and backcrosses. Backcrossing, when occurring, is largely to *P. empetriformis*. Backcrossing to *P. glanduliflora*

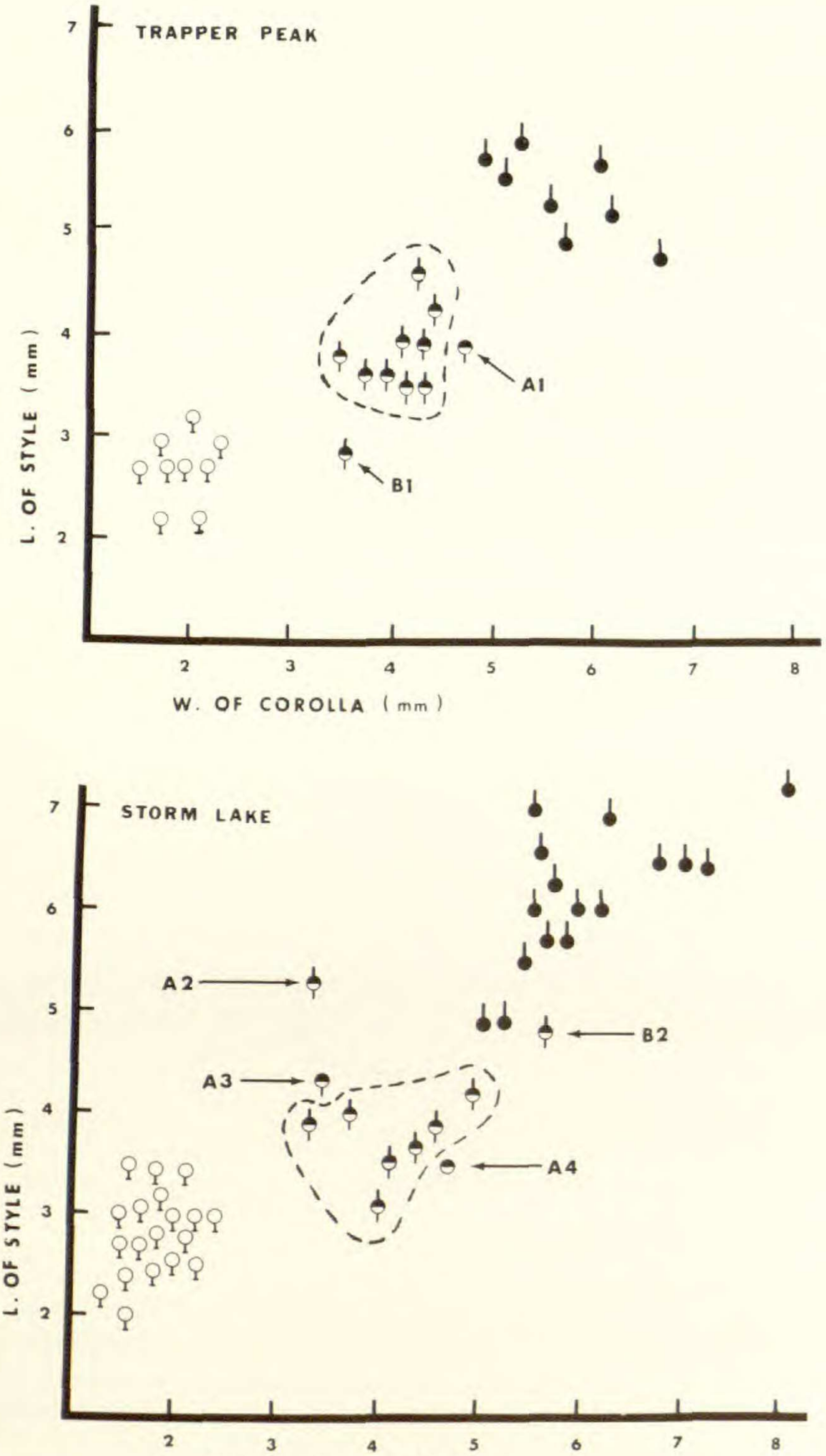
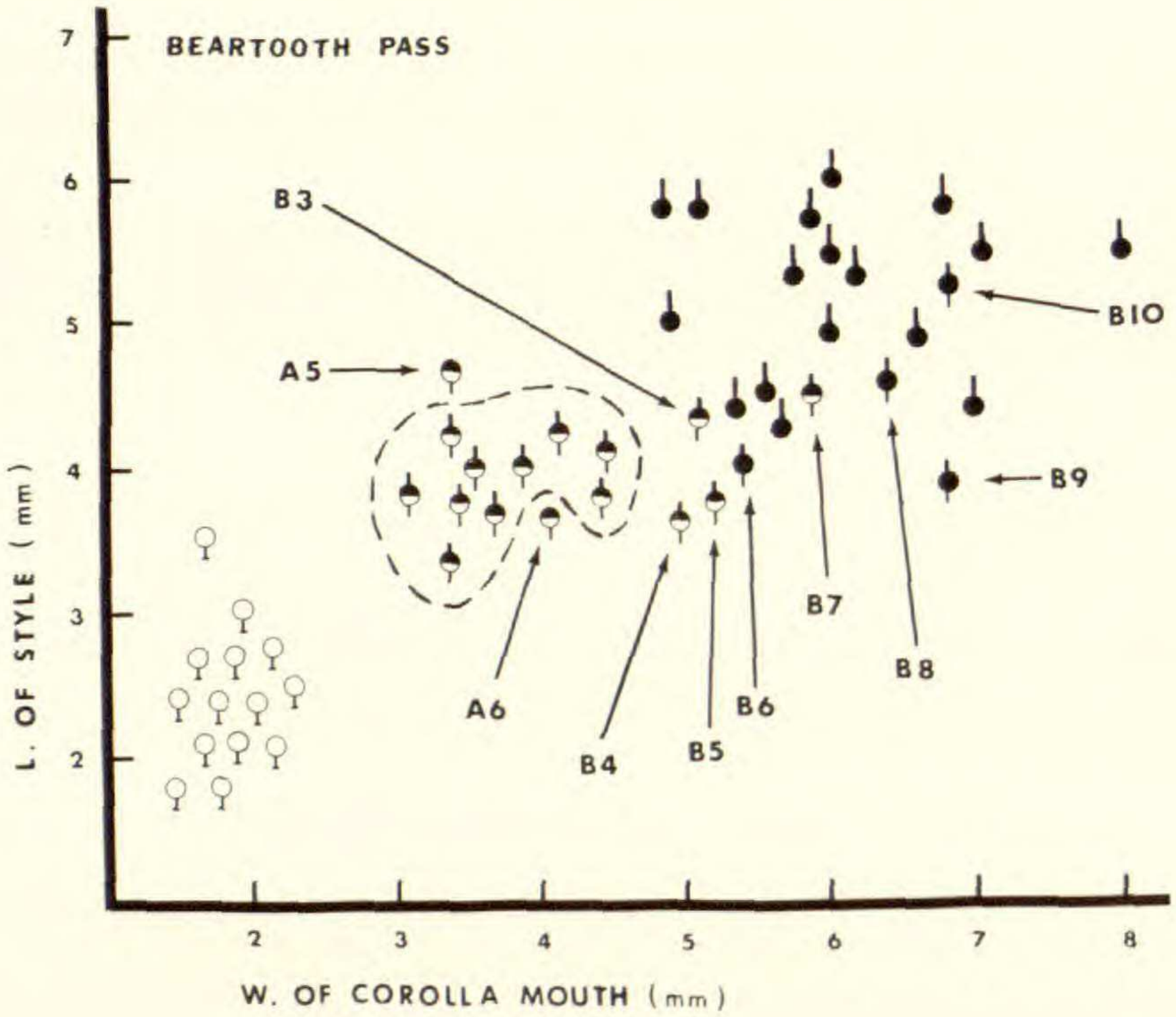


Figure 5. Pictorialized scatter diagrams of variation measured in the Trapper Peak and Storm Lake hybrid populations. Symbols are listed in Table 2. Trapper Peak = *Watson 958* (Table 1); Storm Lake = *Watson 976* (Table 1). Putative F_1 progeny are enclosed within broken lines. Filial segregates are designated as A1–A4. Backcrosses are designated as B1 and B2.



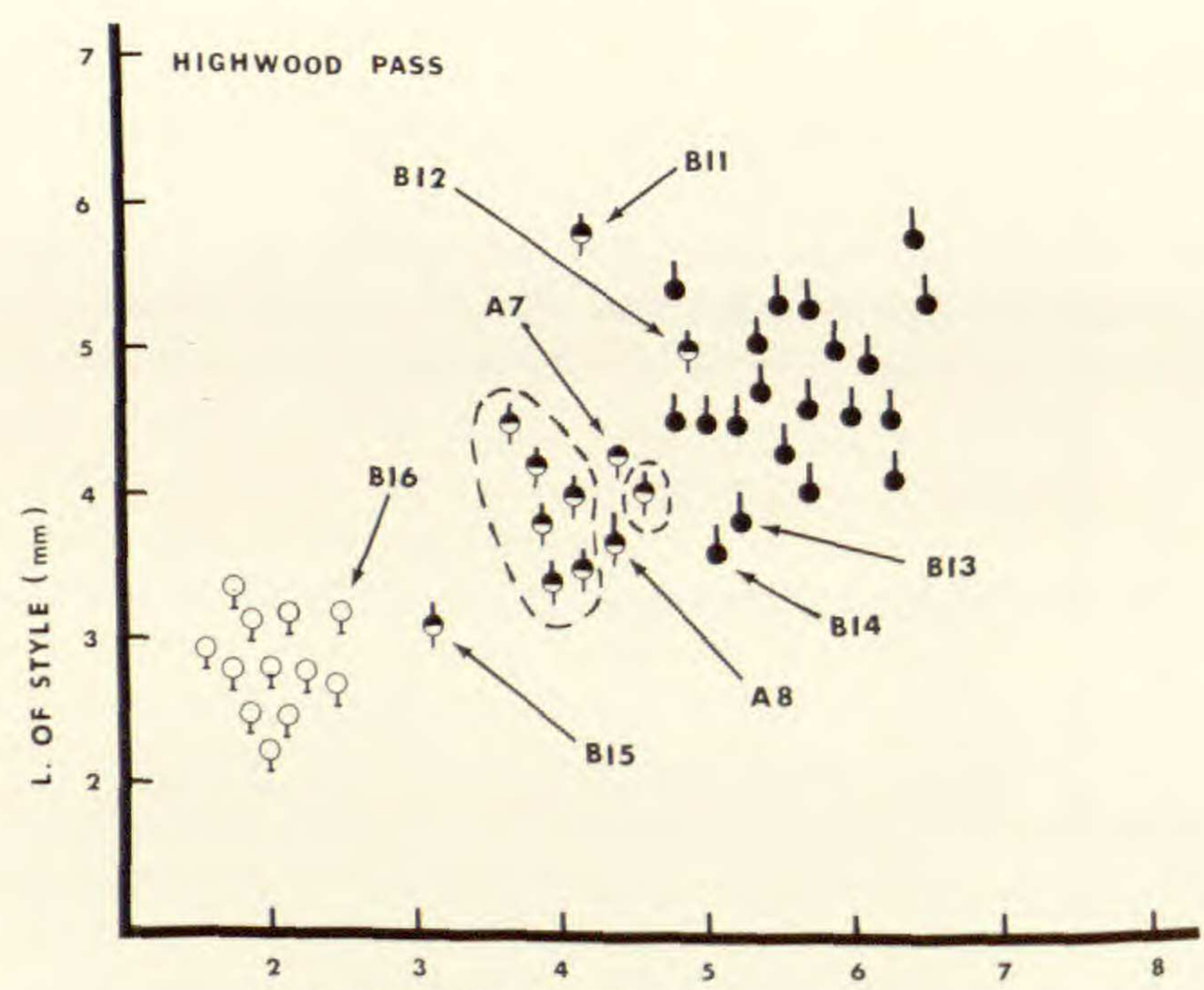


Figure 6. Pictorialized scatter diagrams representative of variation measured in the Beartooth Pass and Highwood Pass hybrid populations. Symbols are listed in Table 2. Beartooth Pass = *Watson 1000* (Table 1); Highwood Pass = *Watson 1009* (Table 1). Putative F_1 progeny are enclosed within broken lines. Filial segregates are designated as A5 – A8. Backcrosses are designated as B3 – B16.

is a rare event. The small amount of gene exchange between the species is restricted to the narrow zones where the two grow intermixed.

While only four sites of hybridization were examined intensively in this investigation, similar population structures probably exist at other locales. This is suggested by herbarium studies in which specimens from 31 other sites of hybridization throughout the range of the taxa were examined. A few of these appeared to be advanced filials or backcrosses to *Phyllodoce empetriformis.* Most seemed to be pure parents or F_1 hybrids. Only two specimens from 1,538 sheets examined were suspected of being backcrosses to *P. glanduliflora.* These were Rydberg's types of *P. hybrida* (*Rydberg & Bessey, 4657:* US, NY).

ACKNOWLEDGMENTS

I wish to thank the curators of the following herbaria for loaning their specimens of *Phyllodoce:* ALA, ALTA, DAO, ID, NY, OSC, UBC, US, UT, UTC, and WTC. This study was made possible by a grant (841–1/u) from The University of Montana Foundation.

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